CLAIMS

I CLAIM:

1. A method of obtaining site-specific gene replacement in a eukaryotic cell, comprising:

a) providing a eukaryotic cell that comprises a receptor construct, wherein the receptor construct comprises a receptor polynucleotide flanked by two or more of a irreversible recombination site (IRS);

b) introducing into the cell a donor construct that comprises a donor polynucleotide flanked by two or more of a irreversible complementary recombination site (CIRS); and

c) contacting the receptor construct and the donor construct with an irreversible recombinase polypeptide;

d) wherein the irreversible recombinase catalyzes recombination between the first and second types of recombination sites and replacement of the receptor polynucleotide with the donor polynucleotide, thereby forming a replacement construct.

2. The method of claim 1, wherein the donor construct is linear.

3. The method of claim 1, wherein the donor construct is a circular vector.

4. The method of claim 1, wherein the donor construct is a chromosome.

5. The method of claim 1, wherein the receptor construct is a chromosome.

6. The method of claim 1, wherein the receptor construct comprises two IRS and the donor construct comprises two CIRS.

7. The method of claim 6, wherein the IRS are inverted with respect to each other and wherein the CIRS are inverted with respect to each other.

8. The method of claim 6, wherein the donor polynucleotide further comprises a promoter operably linked to a gene of interest.

9. The method of claim 6, wherein the receptor construct further comprises a promoter that is adjacent to one of the IRS.

10. The method of claim 9, wherein the promoter is located in the 5 prime direction from the IRS.

11. The method of claim 9, wherein the receptor construct further comprises a second promoter operably linked to a selectable marker.

12. The method of claim 9, wherein the receptor polynucleotide or the donor polynucleotide further comprises a negative selectable marker.

13. The method of claim 9, wherein the receptor polynucleotide or the donor polynucleotide further comprises a nucleic acid encoding the irreversible recombinase polypeptide.

14. The method of claim 13, wherein the receptor polynucleotide comprises the nucleic acid encoding the irreversible recombinase polypeptide.

15. The method of claim 14, wherein the irreversible recombinase polypeptide is a ϕC31 integrase, a coliphage P4 recombinase, a coliphage lambda recombinase, a *Listeria* U153 or A118 phage recombinase, or an actinophage R4 Sre recombinase.

16. The method of claim 15, wherein the irreversible recombinase is a bacteriophage ϕC31 integrase.

17. The method of claim 1, further comprising deleting undesired nucleotide sequences in the replacement construct by contacting the replacement construct with a reversible recombinase, wherein the replacement construct comprises one or more pairs of directly oriented reversible recombination sites (RRS) that are compatible with the reversible recombinase.

18. The method of claim 17, wherein the reversible recombinase is selected from the group consisting of a Cre from phage P1, a FLP of yeast, a Gin recombinase of phage Mu, a R recombinase of a pSR1 plasmid, and a β recombinase from a *Bacillus* phage.

19. The method of claim 17, wherein the receptor construct comprises two IRS and the donor construct comprises two CIRS.

20. The method of claim 19, wherein the donor polynucleotide comprises two of the RRS, which two are oppositely oriented with respect to each other.

21. The method of claim 20, wherein the RRS flank a promoter and a gene of interest.

22. The method of claim 21, wherein the receptor construct further comprises two of the RRS, which two are oppositely oriented with respect to each other.

23. The method of claim 22, wherein the RRS flank a promoter and the receptor polynucleotide as flanked by the two IRS.

24. The method of claim 17, wherein the receptor construct comprises three IRS and the donor construct comprises three CIRS.

25. The method of claim 24, wherein the three IRS consist of two IRS that are identical and one IRS that is non-identical, and wherein the three CIRS consist of two CIRS that are identical and one CIRS that is non-identical.

26. The method of claim 25, wherein the donor polynucleotide further comprises a promoter operably linked to a gene of interest.

27. The method of claim 25, wherein the receptor construct further comprises a promoter that is adjacent to one of the IRS.

28. The method of claim 27, wherein the promoter is located in the 5 prime direction from the IRS.

29. The method of claim 27, wherein the receptor construct further comprises a second promoter operably linked to a selectable marker.

30. The method of claim 27, wherein the receptor polynucleotide or the donor polynucleotide further comprises a negative selectable marker.

31. The method of claim 27, wherein the receptor polynucleotide or the donor polynucleotide further comprises a nucleic acid encoding the irreversible recombinase polypeptide.

32. The method of claim 31, wherein the receptor polynucleotide comprises the nucleic acid encoding the irreversible recombinase polypeptide.

33. The method of claim 32, wherein the irreversible recombinase polypeptide is a ϕC31 integrase, a coliphage P4 recombinase, a coliphage lambda recombinase, a *Listeria* U153 or A118 phage recombinase, or an actinophage R4 Sre recombinase.

34. The method of claim 33, wherein the irreversible recombinase is a bacteriophage ϕC31 integrase.

35. The method of claims 1 or 17, wherein the eukaryotic cell is selected from a mammalian cell or a plant cell.

36. The method of claim 35, wherein the eukaryotic cell is a plant cell.

37. The plant cell produced by the method of claim 36.

38. A plant comprising the plant cell of claim 37.

39. The method of claim 35, wherein the eukaryotic cell is a human cell.

40. A method of producing a transgenic plant, comprising the steps of:

a) providing a receptor plant comprising a chromosomal receptor polynucleotide flanked by two of a irreversible recombination site (IRS);

b) providing a donor plant comprising a chromosomal donor polynucleotide flanked by two of a complementary irreversible recombination site (CIRS);

c) crossing the donor plant the receptor plant to produce a transgenic plant, wherein the transgenic plant expresses an irreversible recombinase polypeptide that catalyzes recombination between the IRS and the CIRS and replacement of the receptor polynucleotide with the donor polynucleotide, thereby forming a chromosomal replacement construct in the transgenic plant.

41. The method of claim 40, wherein the receptor plant is a single copy receptor line.

42. The method of claim 40, wherein the receptor plant and the donor plant are the same species.

43. The method of claim 40, wherein the receptor polynucleotide further comprises a nucleic acid encoding the irreversible recombinase polypeptide.

44. The method of claim 43 further comprising, selecting a progeny of the transgenic plant that does not express the irreversible recombinase polypeptide.

45. The method of claim 40, wherein the chromosomal replacement construct comprises a promoter operably linked to the donor polynucleotide.

46. The method of claim 45, wherein the promoter is derived from the receptor plant.

47. The method of claim 40, wherein the receptor polynucleotide or the donor polynucleotide further comprises a negative selectable marker.

48. The method of claim 40, wherein the IRS are inverted with respect to each other and wherein the CIRS are inverted with respect to each other.

49. The method of claim 40, wherein the irreversible recombinase polypeptide is a ϕC31 integrase, a coliphage P4 recombinase, a coliphage lambda recombinase, a *Listeria* U153 or A118 phage recombinase, or an actinophage R4 Sre recombinase.

50. The method of claim 49, wherein the irreversible recombinase is a bacteriophage ϕC31 integrase.

51. The method of claim 40, further comprising crossing the transgenic plant with a plant comprising a nucleic acid encoding a reversible recombinase wherein the chromosomal replacement construct further comprises one or more pairs of directly oriented reversible recombination sites (RRS) that are compatible with the reversible recombinase.

52. A transgenic plant produced by the method of any of claims 40-51.

53. A method of gene stacking in a cell comprising:

a) providing a cell that comprises a target construct in a chromosome, wherein the target construct comprises a target polynucleotide, two of a reversible recombination site (RRS), wherein the RRS are oppositely oriented with respect to each other and wherein the target polynucleotide comprises at least one irreversible recombination site (IRS);

b) introducing into the cell a first donor construct that comprises a first donor polynucleotide, two of a complementary irreversible recombination site (CIRS) and two RRS that are oppositely oriented with respect to each other;

c) contacting the target construct and the first donor construct with an irreversible recombinase polypeptide that is compatible with each of the IRS and the CIRS, wherein the irreversible recombinase integrates the first donor polynucleotide into the target construct, thereby forming a first chromosomal integration construct;

d) deleting undesired nucleotide sequences in the first chromosomal integration construct by contacting the locus with a reversible recombinase polypeptide compatible with each of the RRS, thereby forming a first chromosomal trait construct;

e) introducing into the cell a second donor construct that comprises two IRS, a second donor polynucleotide and one RRS;

f) contacting the first chromosomal trait construct and the second donor construct with the irreversible recombinase polypeptide, wherein the irreversible recombinase integrates the second donor polynucleotide into the first chromosomal trait construct, thereby forming a second chromosomal integration construct;

g) selecting for a cell containing a second chromosomal integration construct wherein the first donor polynucleotide is adjacent to the second donor polynucleotide;

h) deleting undesired nucleotide sequences in the selected second chromosomal integration construct by contacting the selected second chromosomal integration construct with a reversible recombinase polypeptide compatible with each of the RRS, thereby forming a second chromosomal trait construct;

i) introducing into the cell a third donor construct that comprises two CIRS, a third donor polynucleotide and one RRS;

j) contacting the second chromosomal trait construct and the third donor construct with the irreversible recombinase polypeptide, wherein the irreversible recombinase integrates the third donor polynucleotide into the second chromosomal trait construct, thereby forming a third chromosomal integration construct;

k) selecting for a cell containing a third chromosomal integration construct wherein the second donor polynucleotide is adjacent to the third donor polynucleotide; and

l) deleting undesired nucleotide sequences in the selected third chromosomal integration construct by contacting the selected third chromosomal integration construct with a reversible recombinase polypeptide compatible with each of the RRS, thereby forming a third chromosomal trait construct.

54. The method of claim 53, wherein any one of the donor constructs is a circular vector.

55. The method of claim 53, wherein the receptor construct is a chromosome.

56. The method of claim 53, wherein any one of the donor polynucleotides comprises a gene of interest operably linked to a promoter.

57. The method of claim 56, where the donor polynucleotide further comprises a selectable marker.

58. The method of claim 57, wherein the receptor construct further comprises a polynucleotide encoding an irreversible recombinase polypeptide.

59. The method of claim 58, wherein the receptor polynucleotide comprises the nucleic acid encoding the irreversible recombinase polypeptide.

60. The method of claim 59, wherein the irreversible recombinase polypeptide is a ϕC31 integrase, a coliphage P4 recombinase, a coliphage lambda recombinase, a *Listeria* U153 or A118 phage recombinase, or an actinophage R4 Sre recombinase.

61. The method of claim 60, wherein the irreversible recombinase is a bacteriophage ϕC31 integrase.

62. The method of claim 56, wherein the receptor construct further comprises a promoter operably linked to a selectable marker.

63. The method of claim 62, wherein the selectable marker is a negative selectable marker.

64. The method of claim 53, wherein the eukaryotic cell is a plant cell or a mammalian cell.

65. The method of claim 64, wherein the eukaryotic cell is a plant cell.

66. The method of claim 64, wherein the eukaryotic cell is a human cell.